UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

PREVENTION

OFFICE OF CHEMICAL SAFETY AND POLLUTION

MEMORANDUM

DATE: September 30, 2014

SUBJECT: **BENZOVINDIFLUPYR:** Report of the Cancer Assessment Review Committee

PC Code: 122305
Decision No.: N/A
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Risk Assessment Type: Cancer Assessment
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FROM: Jess Rowland, Co-Chair
Karlyn Middleton, Co-Chair
Cancer Assessment Review Committee
Health Effects Division (7509P)

TO: William Irwin, PhD, DABT, Toxicologist
Michael Metzger, Branch Chief (RAB V and VII)
Health Effects Division (7509P)

The Cancer Assessment Review Committee (CARC) met on July 30, 2014 to evaluate the cancer classification of benzovindiflupyr in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). Attached please find the final Cancer Assessment Document.

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

BENZOVINDIFLUPYR (SOLATENOL)

PC CODE 122305

September 24, 2014

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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I. EXECUTIVE SUMMARY

On July 30, 2014, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs evaluated the carcinogenic potential of the new active ingredient, benzovindiflupyr. The toxicological database is being jointly reviewed by the Canadian Pest Management Regulatory Agency (PMRA) and the U.S. Environmental Protection Agency (EPA). William Irwin of Risk Assessment Branch V presented the chronic toxicity/carcinogenicity study in Wistar rats and the carcinogenicity study in CD-1 mice. He also discussed the toxicology, metabolism, and mutagenicity studies, as well as structure-activity relationships and mode of action information.

Benzovindiflupyr was administered in the diet to male and female Wistar rats at 0, 25, 100, 600 (male) or 400 (female) ppm for 24 months. This administration is equivalent to 0, 1.21, 4.88 and 30.17 mg/kg/day in males and 0, 1.65, 6.66 and 27.44 mg/kg/day for females. There was no evidence of carcinogenicity in female rats. In males at the high dose, there was a statistically significant trend and pair-wise significance ($p<0.05$) for increased thyroid follicular cell adenomas and the combined follicular cell adenomas and/or carcinomas. The incidences for adenomas (18%) exceeded the historical control range (2-11%) of the testing laboratory. Male rats at the high dose also had increased incidence of granular cell tumors of the brain (10%) when compared to controls (4%). Although there was no pair-wise significance, the CARC determined that the brain tumors are treatment-related since there was a positive trend ($p<0.01$) and the incidences exceeded the historical control range (0-6%). The Committee noted that the tumors occurred late in the study (first tumor was seen at Study Week 91). The CARC determined that the dose levels tested were adequate to assess the carcinogenic potential of benzovindiflupyr in rats, based on the presence of systemic toxicity (decrements in body weights and clinical signs in both sexes) and the occurrence of tumors at the high dose.

There was no evidence of carcinogenicity in male and female CD-1 mice fed diets containing benzovindiflupyr at 0, 20, 60, or 200 ppm (equivalent to 0, 2.62, 7.55 and 26.18 mg/kg/day for males and 0, 2.89, 8.67 and 29.26 for females) for 18 months. The CARC determined that the doses tested were adequate to assess the carcinogenic potential of benzovindiflupyr, based on clinical signs of toxicity (piloerection and rolling gain) seen in females at the high dose and the presence of hyperplasia of the colon and cecum in both sexes at the high dose.

Mechanistic studies were submitted to support a proposed non-genotoxic mode of action (MoA) for the thyroid follicular cell adenomas observed in male rats. The hypothalamus releases thyrotropin-releasing hormone (TRH), which stimulates the anterior pituitary gland to secrete thyroid stimulating hormone (TSH). TSH induces the thyroid gland to produce and release thyroxine (T4) and triiodothyronine (T3). The expression of TSH and TRH is controlled through a negative-feedback process that is very sensitive to circulating T4 and T3 levels.

The key events for the MoA include: 1) induction of UDPGT, 2) increased T3/T4 clearance and decreased plasma T3/T4 levels, 3) increased TSH levels, and 4) increased thyroid follicular cell proliferation, which leads to increased thyroid follicular cell tumors. The mode of action was adequately supported by studies that clearly identified the sequence of key events, dose-response concordance and temporal relationship for the thyroid tumors. Alternate modes of actions for thyroid tumors were also considered, but were not supported by the available data.

The CARC, in accordance with the Agency's 2005 Guidelines for Carcinogen Risk Assessment, classified benzovindiflupyr as showing "*Suggestive Evidence of Carcinogenic Potential*" based on the presence of granular cell tumors of the brain in male rats. The CARC concluded that a non-genotoxic mode of action for thyroid tumors observed in male rats has been established as a result of upregulation of UDPGT, increased clearance of T3 and T4 hormones and increased TSH levels, resulting in increased thyroid cell proliferation, which progress to form thyroid tumors. There was no evidence of carcinogenicity in female rats or in male or female mice. In addition, there is no concern for mutagenicity.

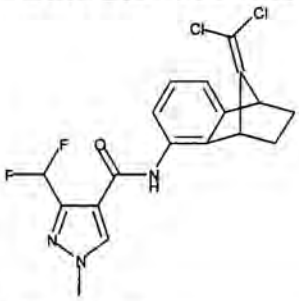
Quantification of carcinogenicity is not required. When there is suggestive evidence of carcinogenicity, the Agency does not attempt a dose-response assessment as the nature of the data generally would not support one. Therefore, the Agency has determined that quantification of risk using a non-linear approach (i.e., RfD) will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to benzovindiflupyr.

II. INTRODUCTION

On July 30, 2014 the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of benzovindiflupyr.

III. BACKGROUND INFORMATION

Benzovindiflupyr (Solatenol; SYN545192) is a new broad-spectrum systemic fungicide of the carboxamide group (FRAC Group 7). It blocks cell respiration in the fungus by inhibiting succinate dehydrogenase (mitochondrial respiration Complex II), thus blocking electron transport. Benzovindiflupyr is used on barley, blueberries, canola, corn, cotton, cucurbits, oats, peanuts, pome fruit, rye, triticale, and wheat. Benzovindiflupyr has also been proposed for use on turf, parks, athletic fields, golf courses, sod farms, nurseries and ornamental plantings. Its structure and other pertinent information are depicted in Table 1.

Table 1: Structure and Chemical Information for Benzovindiflupyr.	
Chemical structure:	
Empirical formula:	$C_{18}N_3OF_2Cl_2H_{16}$
Common name:	Solatenol
Company code:	SYN545192
IUPAC name:	*N*-[(1*RS*,4*SR*)-9-(Dichloromethylidene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxamide
CAS name:	*N*-[9-(dichloromethylene)-1,2,3,4-tetrahydro-1,4-methanonaphthalen-5-yl]-3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide
CAS no.:	1072957-71-1
PC Code	122305

The pesticidal mode of action of carboxamide fungicides is through inhibition of succinate dehydrogenase (SDH), which is a functional part of the tricarboxylic acid cycle (TCA), the mitochondrial electron transport chain and oxidative phosphorylation. Mitochondria typically supply ~90% of the ATP in eukaryotes for cellular energy demands (i.e. protein synthesis, DNA synthesis, ion pumping, etc). Benzovindiflupyr has been demonstrated to inhibit SDH in mammalian heart mitochondria.

IV. EVALUATION OF CARCINOGENICITY STUDIES

1. *Combined Chronic Toxicity/Carcinogenicity Study with Benzovindiflupyr in Wistar Rats*

Reference: Mackay C, 2012a. SYN545192: 104 week rat dietary carcinogenicity study with combined 52 week toxicity study. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Laboratory Report No. 30797, 02 February 2012. Unpublished. (Syngenta File No. SYN545192_10183). MRID 48604446

A. Experimental Design

In a combined chronic toxicity and carcinogenicity study with SYN545192 (purity 97.0%), four groups of Han Wistar rats/sex were assigned to the carcinogenicity study and fed diets containing 0, 25, 100 or 600 (males), 400 (females) ppm SYN545192 for at least 104 consecutive weeks. These doses corresponded to 0, 1.21, 4.88 and 30.17 mg/kg/day in males and 0, 1.65, 6.66 and 27.44 mg/kg/day for females. In addition, a chronic toxicity study (comprising a further 4 groups

of 12 rats/sex) was included and dosed in an identical fashion for a period of 52 consecutive weeks.

B. *Survival Analysis*

There was no treatment-related effect on survival in either sex at any dose level.

C. Discussion of Tumor Data

There was no evidence of carcinogenicity in female rats, whereas thyroid follicular cell and brain tumors were seen in male rats only at the high dose. The statistical analyses of the tumors in the male rats were based upon Fisher's Exact Test and the Exact Test for Trend (L. Brunsman, TXR0056999, 07/02/2014).

For the thyroid tumors, there was a significant trend and pair-wise significance at 600 ppm for the follicular cell adenomas and combined adenomas/carcinomas. The incidences for adenomas (18%) exceeded the historical control range (2-11%) of the testing laboratory. Only a single carcinoma was found in the control group and 100 ppm group (Table 2).

For the brain tumors, there was no statistical significance when compared to the concurrent controls, however, the incidence (10%) at the high dose exceeded the historical control range (0-6%) of the testing laboratory (Table 3).

Table 2: Thyroid Follicular Cell Tumors in Male Rats Following[a] Dietary Administration of Benzovindiflupyr.

Tumors	0 ppm	25 ppm	100 ppm	600 ppm
Adenomas (%)	1/51 (2)	4[a]/52 (8)	5/52 (10)	9/51 (18)
p =	0.0072**	0.1874	0.1069	0.0079**
Carcinomas (%)	1[b]/51 (2)	0/52 (0)	1[b]/52 (2)	0/51 (0)
p =	0.3744	1.0000	0.7573	1.0000
Combined (%)	2/51 (4)	4/52 (8)	6/52 (12)	9/51 (18)
p =	0.0171*	0.3484	0.1410	0.0257*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a]First adenoma observed at week 97, dose 25 ppm. TXR 0055854.
[b]First carcinoma observed at final sacrifice simultaneously in dose groups 0 and 100 ppm.
* Statistically significant difference from control group mean, $p<0.05$
** Statistically significant difference from control group mean, $p<0.01$
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.

Table 3: Brain Tumors in Male Rats Following Dietary Administration of Benzovindiflupyr.				
Tumors	**0 ppm**	**25 ppm**	**100 ppm**	**600 ppm**
Granular Cell Tumors (%)	2[a]/51 (4)	0/52 (0)	0/52 (0)	5/51 (10)
p =	0.0110**	1.0000	1.0000	0.2182

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a]First granular cell tumor observed at week 91, dose 0 ppm. TXR 0056999.
* Statistically significant difference from control group mean, $p<0.05$
** Statistically significant difference from control group mean, $p<0.01$
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.

D. Non-neoplastic findings

The non-neoplastic lesions are presented in Table 4. In the liver, eosinophilic cell foci were noted in males at 600 ppm. Centrilobular hypertrophy was noted at doses ≥ 100 ppm in males and at 400 ppm in females. There were increases in centrilobular hepatocyte pigmentation in females at 400 ppm and hepatocyte vacuolation in males at 600 ppm. Focal C-cell hyperplasia of the thyroid gland was seen in females at 600 ppm.

Table 4: Non-Neoplastic Findings in Rats Following Dietary Administration of Benzovindiflupyr.								
	Dietary Concentration (ppm)							
	Males				**Females**			
	0	**25**	**100**	**600**	**0**	**25**	**100**	**400**
Liver (Number of animals)	(52)	(52)	(52)	(52)	(52)	(52)	(52)	(52)
Liver - Eosinophilic cell focus (total)	1	5	4	**15****	2	0	3	5
- minimal	0	2	0	**2**	0	0	2	1
- mild	1	2	4	**6**	2	0	1	2
- moderate	0	1	0	**6***	0	0	0	1
- marked	0	0	0	**1**	0	0	0	1
Liver - Centrilobular hypertrophy (total)	0	1	**8****	**13****	0	2	5	**36****
- minimal	0	0	**1**	**2**	0	1	2	**3**
- mild	0	1	**6***	**11****	0	1	3	**33****
- moderate	0	0	**1**	**0**	0	0	0	**0**
Liver - Hepatocyte pigment centrilobular (total)	0	0	0	0	0	0	0	**3**
- minimal	0	0	0	0	0	0	0	1
- mild	0	0	0	0	0	0	0	1
- moderate	0	0	0	0	0	0	0	1

Table 4: Non-Neoplastic Findings in Rats Following Dietary Administration of Benzovindiflupyr.								
	Dietary Concentration (ppm)							
	Males				Females			
	0	25	100	600	0	25	100	400
Liver - Hepatocyte vacuolation (total)	2	5	5	9	0	2	5	0
- minimal	0	0	2	3	0	0	3	0
- mild	2	2	1	5	0	2	2	0
- moderate	0	1	2	1	0	0	0	0
- marked	0	1	0	0	0	0	0	0
- severe	0	1	0	0	0	0	0	0
Thyroid (Number of animals)	(52)	(52)	(52)	(52)	(50)	(52)	(52)	(52)
Thyroid - Focal C-cell hyperplasia Total	11	3*	2*	5	3	5	4	9

* Statistically significant difference from control group mean, $p<0.05$ (Fisher's Exact Test), MRID 48604446

** Statistically significant difference from control group mean, $p<0.01$ (Fisher's Exact Test)

E. Adequacy of Dosing for Assessment of Carcinogenicity

The doses tested were considered to be adequate and not excessive in both genders, based on the decreases in body weights (males: 11% and females: 25%), clinical signs (lack of response to tactile stimulus, hunched or held low body position, piloerection, staining on fur and thin appearance, slow reaction to tail flick, and rolling gait) in both sexes, and presence of thyroid and brain tumors at the high dose in male rats.

2. Carcinogenicity Study in Mice

Reference: Mackay C, 2012b. SYN545192 - 80 week mouse dietary carcinogenicity study. Charles River, Tranent, Edinburgh, EH33 2NE, UK. Laboratory Report No. 32209, 06 February 2012. Unpublished. (Syngenta File No.SYN545192_10189). MRID 48604448

A. Experimental Design

In a carcinogenicity study, four groups of 50 CD-1 mice/sex/ group were fed diets containing 0, 20, 60 or 200 ppm benzovindiflupyr (97%) for 80 weeks. These dose levels were equivalent to 0, 2.62, 7.55 and 26.18 mg/kg/day for males and 0, 2.89, 8.67 and 29.26 for females.

B. Survival analysis

There was no treatment-related effect on survival in either sex at any dose level.

C. Discussion of Tumor Data

There was no evidence of carcinogenicity in male or female mice. (L. Brunsman, TXR0056999, 07/02/2014). There were no significant trends and no significant pair-wise comparisons of the dosed groups with the controls for uterine leiomyomas or leiomyosarcomas, or for ovarian tubulostromal adenomas. The Harderian gland tumors seen in male mice were considered to be treatment related but not relevant to humans and were not considered in the weight of the evidence evaluation.

D. Non-Neoplastic Lesions

As shown in Table 5, non-neoplastic findings were limited to the colon and caecum of the large intestine of both sexes. Hyperplasia in the two organs was increased in incidence and severity at the high dose.

Table 5: Non-Neoplastic Findings in Mice Following Dietary Administration of Benzovindiflupyr.

	Dietary Concentration of Benzovindiflupyr (ppm)							
	Males				Females			
	0	20	60	200	0	20	60	200
Colon Hyperplasia Incidence								
(no of animals)	**(49)**	**(50)**	**(50)**	**(49)**	**(48)**	**(49)**	**(49)**	**(48)**
Minimal	0	1	0	3	0	0	0	3
Mild	1	0	0	6	0	0	0	6*
Moderate	0	1	0	3	0	0	0	1
Marked	0	0	0	1	0	0	0	0
Total Incidence	1	2	0	13**	0	0	0	10**
Cecum Hyperplasia Incidence								
(no of animals)	**(49)**	**(50)**	**(49)**	**(50)**	**(48)**	**(49)**	**(49)**	**(48)**
Minimal	0	0	0	1	0	0	0	0
Mild	0	0	1	3	0	0	0	2
Total Incidence	0	0	1	4	0	0	0	2

*p<0.05, **p<0.01; [a] Data obtained from page 32 of the study report in MRID 48604448

E. Adequacy of Dosing for Assessment of Carcinogenicity

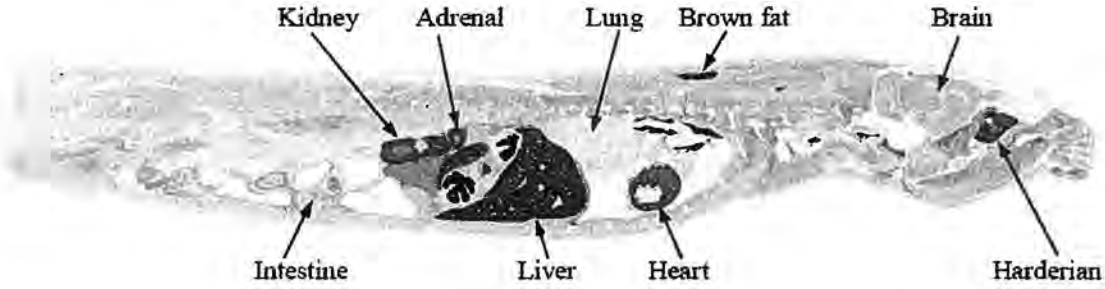
The doses tested were considered to be adequate and not excessive in both sexes based on clinical signs of toxicity (piloerection and rolling gain) seen in females at the high dose and the presence of hyperplasia in the colon/cecum in both genders at the highest dose.

V. TOXICOLOGY

a. Metabolism

In a rat metabolism study (MRID # 48604426), oral Absorption was similar in male and female rats following low and high doses (79-81% and 61-62%, respectively). During the first 48 hours post dose, the majority of the administered radioactivity had been excreted (97% and 89% following 1 mg/kg and 90% and 86% following 40 mg/kg in males and females, respectively). The systemic oral bioavailability was essentially complete in both genders.

Whole body auto-radiography using both radiolabels of benzovindiflupyr, total radioactivity was extensively distributed throughout the body by the first sampling time of 5 hours in males and 1 hour in females and had declined markedly in both sexes by 72 hours post dose. There were no clear differences in tissue distribution profiles between the labels nor were any pronounced gender or dose differences apparent. The highest tissue concentrations were present in the Harderian gland and liver, with lower concentrations in the adrenal gland, heart, brown fat, and kidney.



Benzovindiflupyr was extensively metabolized in the rat giving rise to at least 8 types of metabolites (*e.g.* desmethyl, hydroxy, dihydroxy, desmethyl hydroxy, desmethyl dihydroxy, ring-open, glucuronide conjugate, and sulphate conjugate). Demethylation, bicyclo ring hydroxylation, and phenyl ring hydroxylation together accounted for a major proportion of the dose (35%-60% dose). There were little differences with dose or between genders.

Irrespective of dose or sex, following a single oral dose of 1 or 40 mg/kg of benzovindiflupyr radioactivity was distributed throughout the body and was rapidly eliminated with the majority being excreted within the first 72 hours post dose (95 and 91% following the low dose and 97 and 91% following the high dose in males and females, respectively). The predominant route of elimination was *via* the feces.

Following daily oral doses of 1 mg/kg, benzovindiflupyr appeared to be approaching steady state concentrations by the end of the 14 day dosing period. Following the cessation of dosing, all tissue concentrations steadily declined. Following the liver and kidney, the adrenals and thyroid had the greatest concentrations of radioactivity. The total tissue and carcass residues at the final sampling time accounted for less than 0.2% of the total radio-labeled dose administered (63 days after the last dose on Day 14). The terminal phase half-life for tissue depletion ranged from 2.5 days in plasma and 69 days in the testes.

b. Mutagenicity

Benzovindiflupyr was negative in the submitted genetic toxicity studies (Table 6). All of the studies were classified as acceptable/guideline and satisfy the requirement for genotoxicity data. Based on the results of the genetic toxicology studies, it was concluded that there is no mutagenic concern for benzovindiflupyr.

Table 6: Benzovindiflupyr Genotoxicity Results.

870.5100 **Bacterial Reverse Mutation Test**	**Salmonella Typhimurium and E. Coli Reverse Mutation Assay** (2011, MRID 48604442) 0, 3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate	**Negative**
870.5300 **In Vitro Mammalian Cell Gene Mutation Test**	**Cell Mutation Assay at the Thymidine Kinase Locus (TK +/-) in Mouse Lymphoma L5178Y Cells** (2010, MRID 48604444) Experiment I- without S9 mix: 2.5; 5.0; 10.0; 15.0; and 20.0 µg/mL & with S9 mix: 5.0; 10.0; 20.0; 30.0; and 40.0 µg/mL Experiment II- without S9 mix: 5.0; 10.0; 20.0; and 30.0 µg/mL & with S9 mix: 10.0; 20.0; 30.0; 40.0; and 50.0 µg/mL Experiment III- without S9 mix: 2.5; 5.0; 10.0; 20.0; and 30.0 µg/mL	**Negative**
870.5375 **In Vitro Chromosome Aberration Test**	**Chromosome Aberration Test in Human Lymphocytes *In Vitro*** (2010, MRID 48604443) Experiment I- with & without S9 mix: 3.1, 5.5, 9.6, 16.9, 29.5, 51.7, 90.5, 158.3, 277.1, 484.9, 848.6, 1485.0 µg/mL Experiment II- without S9 mix 0.06, 0.11, 0.19, 0.34, 0.60, 1.04, 1.83, 3.2, 5.6, 9.8, 17.14, 30.0 µg/mL & with S9 mix: 1.3, 2.5, 5.0, 7.5, 10.0, 15.0, 20.0, 30.0 µg/mL	**Negative**
870.5395 **In Vivo Micronucleus Test**	**Micronucleus Test in Bone Marrow Cells of Wistar (Han) Rats** (2011, MRID 48604445) 0, 43.8, 87.5, 175.0 mg/kg/day in males 0, 75 mg/kg/day in females	**Negative**

c. Structure-Activity Relationship

Benzovindiflupyr is a member of the pyrazole carboxamide class of mitochondrial succinate dehydrogenase (SDH)-inhibitor fungicides. The CARC previously reviewed two SDH inhibitors, sedaxane and isopyrazam. In accordance with the EPA's *Final Guidelines for Carcinogen Risk Assessment* (March, 2005), sedaxane and isopyrazam are classified as "Likely to be Carcinogenic to Humans". For sedaxane, this classification is based on the presence of liver and thyroid tumors in male rats, uterine tumors in female rats and liver tumors in male mice. For isopyrazam, this classification was based on the presence of thyroid follicular cell tumors in male rats, and liver and uterine tumors in female rats.

d. Subchronic Toxicity

i. Subchronic Toxicity: Repeated Dose 28-day Oral Toxicity Study in Rats

In a 28-day rat study (MRID 48604433), the test material was administered continuously throughout the treatment period by dietary admixture on a parts per million (ppm) basis to three groups, each of five male and five female Wistar HanTM:HsdRccHanTM: WIST strain rats, for twenty-eight consecutive days, at dietary concentrations of 100, 400 and 1200 ppm (equivalent to a mean achieved dosage of 9, 36 and 99 mg/kg/day). A control group of five males and five females were treated with basal laboratory diet alone. There were no unscheduled deaths during the study, and no adverse clinical signs were observed. There were no treatment-related changes in the behavioral or sensory reactivity assessments and no changes in the functional performance parameters measured. There was a reduction in body weight gain and food consumption in those animals dosed at 1200 ppm. The study NOAEL is 36 mg/kg/day (M/F) with the lowest observed adverse effect level (LOAEL) of 99 mg/kg/day (M/F), based on decreased body weight, decreased body weight gain, hypoglycemia, and decreased serum potassium levels.

ii. Subchronic Toxicity: Repeated Dose 90-day Oral Toxicity Study in Rats

In a sub-chronic toxicity study (MRID 48604436), benzovindiflupyr was administered to Han Wistar 10 rats/sex/dose in the diet at dose levels of 0, 100, 750 or 1500 ppm (0, 7.6, 53.8 and 108.7 mg/kg/day for males and 0, 8.2, 58.8 and 108.8 mg/kg/day for females) for 90 days. The study NOAEL is 7.6/8.2 mg/kg/day (M/F) with a LOAEL of 53.8/58.8 mg/kg/day (M/F), based on reduction in body weight, body weight gain, food consumption, and food utilization; a decrease in core body temperature and glucose in females; increased liver weight in females; hepatic centrilobular hypertrophy of the liver in males; and increased urea in males.

iii. Subchronic Toxicity: Repeated Dose 28-day Oral Toxicity Study in Mice

The purpose of the study was to assess the potential toxicity of the benzovindiflupyr in the mouse after oral administration via the diet for 4 weeks (MRID 48604435). Groups of 5 male and 5 female CD-1 mice were fed diets containing 0, 100, 300 or 500 ppm (0, 15.6, 47.4 and 81.8 mg for males and 0, 19.0, 57.9 and 91.5 mg/kg/day for females) benzovindiflupyr for a period of at least 28 days. Additional satellite groups of 7 control male and 7 female animals, and 15 male and 15 female animals at all other dose levels, were incorporated onto the study for toxicokinetic sampling and analysis. The animals were monitored regularly for viability and for signs of ill health or reaction to treatment. Body weights and food consumption were measured and recorded at predetermined intervals from pretrial up until the completion of treatment. Blood samples were collected from all Main Study animals prior to necropsy for hematology and clinical chemistry analysis. Toxicokinetic blood samples were collected from designated Satellite Study animals over Days 25 and 26 of the study. The study NOAEL is 15.6/19.0 mg/kg/day (M/F) with a LOAEL of 47.4/57.9 mg/kg/day (M/F) based on decreased body weight and lower body weight gain. Tubulointerstitial nephritis was observed in the kidneys in both sexes at 81.8 mg/kg/day in males and 91.5 mg/kg/day in females.

iv. Sub-chronic Toxicity: Repeated Dose 90-day Oral Toxicity Study in Mice

In a sub-chronic toxicity study (MRID 48604437), benzovindiflupyr (97 % a.i.) was administered to 10 CD-1 mice/sex/dose in diet at dose levels of 0, 100, 300 or 500 ppm (0, 17.0, 55.6 and 97.9 mg/kg/day for males and 0, 20.9, 59.6 and 102.8 mg/kg/day for females) for 90 days. Additional satellite groups of 4 male and 4 female animals were incorporated onto the study for toxicokinetic sampling and analysis. The study NOAEL is 17.6/20.9 mg/kg/day (M/F). The LOAEL is 55.6/59.6 mg/kg/day (M/F), based on an initial body weight loss for up to 7 days (which led to 2 males from the high dose satellite group being terminated in extremis), group mean body weights lower during the initial phases of the study, clinical chemistry changes, and higher incidence of soft feces in males from day 31 onward. In males there was decreased triglycerides and distended large intestine in one animal of each sex.

e. Chronic Toxicity

i. Chronic Toxicity: Repeated Dose 2-Year Oral Toxicity Study in Rats

In a combined chronic toxicity and carcinogenicity study (MRID 48604446), four groups of Han Wistar rats/sex were assigned to the carcinogenicity study and fed diets containing 0, 25, 100 or 600 (males), 400 (females) ppm benzovindiflupyr (97% pure) for at least 104 consecutive weeks. These doses were equivalent to 1.21, 4.88 and 30.17 mg/kg/day in males and 0, 1.65, 6.66 and 27.44 mg/kg/day in females. In addition, a chronic toxicity study (comprising a further 4 groups of 12 rats/sex) was included and dosed in an identical fashion for a period of 52 consecutive weeks.

Clinical observations, body weight, food consumption, hematology, coagulation, clinical chemistry, organ weights, gross necropsy, and histopathology were assessed in all groups. Urinalysis and ophthalmoscopy were assessed in the carcinogenicity groups only. A detailed functional observation battery assessment was made in the toxicity groups only. All surviving carcinogenicity and toxicity study animals were terminated after the completion of 104 or 52 weeks of treatment respectively and underwent a detailed necropsy examination with a comprehensive histopathological evaluation.

Body weight and body weight gains were decreased throughout treatment at 600/400 ppm in males and females, respectively. Food consumption was decreased throughout treatment at 400 ppm in females and sporadically in males at 600 ppm. Food utilization was decreased in both sexes at 600/400 ppm. In the clinical and detailed function observations, there was a slight increase in animals exhibiting no reaction to the tactile stimulus at 600/400 ppm and females at 400 ppm exhibited a slower tail flick response and were found to exhibit hunched/held low body posture, piloerection, staining on fur, thin appearance and rolling gait.

Changes to the liver occurred at 600 ppm in males and 400 ppm in females. Relative liver weights were increased in males following 104 weeks of treatment. In the toxicity phase, males and females exhibited centrilobular hypertrophy, and females exhibited centrilobular hepatocyte pigmentation. In the carcinogenicity phase, males and females exhibited centrilobular hypertrophy; males exhibited pale foci grossly and eosinophilic cell foci and hepatocyte vacuolation histopathologically; and females exhibited an increase in hepatocyte centrilobular pigment.

Other microscopic changes were seen in 400 ppm females following 104 weeks of treatment and consisted of increased tubular cell pigment deposits in the kidneys, focal c-cell hyperplasia of the thyroid, lobular hyperplasia of the mammary glands and pigmented macrophages in the spleen. Neoplastic changes consisted of an increase in thyroid follicular cell adenomas in 600 ppm males.

The study NOAEL is 4.88/6.66 mg/kg/day (M/F) with a LOAEL of 30.17/27.44 (M/F) mg/kg/day, based on decreased body weights, body weight gains, food consumption, food utilization, lack of response to tactile stimulus and liver changes in both genders; hunched or held low body position, piloerection, staining on fur and thin appearance, slow reaction to tail flick and rolling gait, and non-neoplastic lesions in both sexes.

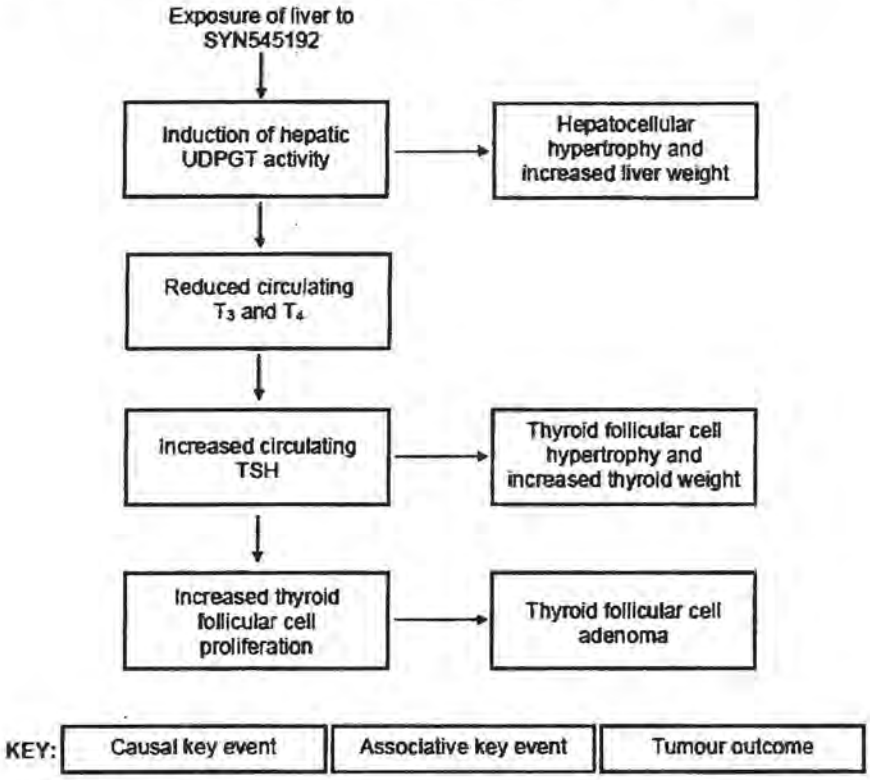
ii. Carcinogenicity Mouse (78 Weeks)

In a carcinogenicity study (MRID 48604448), four groups of 50 CD-1 mice/sex/group were fed diets containing 0, 20, 60 or 200 ppm benzovindiflupyr (97% pure) for at least 80 consecutive weeks. The animals were monitored regularly for viability and for signs of ill health or reaction to treatment. Body weights and food consumption were measured and recorded at pre-determined intervals from pre-trial up until the completion of treatment. Blood samples for hematology were also collected from all surviving animals prior to terminal kill at week 80. Blood films were made from all surviving animals during week 52/53. All surviving animals were terminated after the completion of 80 weeks of treatment and underwent a detailed necropsy examination with a comprehensive histological evaluation. There were no adverse effects on survival, body weight, body weight gain, food or water consumption, hematology, organ weights, or gross pathology. Females dosed at 200 ppm exhibited increased incidences of piloerection and rolling gait. Males and females at 200 ppm exhibited increased mucosal hyperplasia of the colon and cecum.

The study NOAEL is 7.55/8.67 mg/kg/day (M/F) with a LOAEL of 26.18/29.26 mg/kg/day (M/F) based on hyperplasia of the colon/cecum in males and females, with piloerection and rolling gait in females.

VI. Mode of Action Studies

Figure 1: Mode of action hypothesis for induction of thyroid follicular cell tumors in male Han Wistar rats by benzovindiflupyr (MRID 48604586).



The submitted mechanistic/MOA studies were conducted to support key events in the MOA for thyroid tumors in rats following exposure to benzovindiflupyr, as well as to characterize the dose- and temporal-response relationships for each of the key events. As thyroid tumors occurred only in the male rat, the mechanistic studies reported in the MOA document focused primarily on thyroid tumors in male rats to reduce the number of animals employed to generate the data to support the proposed MOA of benzovindiflupyr. Most of the thyroid tumor MOA information presented in this discussion section is derived from the submissions MRIDs 48604553, 48604554, 48604555, 48604556, and 48604586.

a. Thyroid Tumor MOA and Key Events

A consequence of increased liver metabolism via UDPGT upregulation in rodents is increased elimination of thyroid hormones, which are produced by the thyroid and monitored by the hypothalamus-pituitary-thyroid axis. The hypothalamus releases thyrotropin-releasing hormone (TRH), which stimulates the anterior pituitary gland to secrete thyroid stimulating hormone (TSH). TSH induces the thyroid gland to produce and release thyroxine (T4) and triiodothyronine (T3). The expression of TSH and TRH is controlled through a negative-feedback process that is very sensitive to circulating T4 and T3 levels. When the liver is exposed to high doses of an UDPGT substrate, the liver increases in size and the production of those enzymes capable of detoxifying the compound also increases. Some of these detoxification enzymes, in particular the Phase 2 enzymes UDPGTs and sulfotransferases, conjugate the inducer with a glucuronide or a sulfate moiety, respectively. This conjugation allows an easier elimination of the compound *via* urinary and biliary clearance (Klaassen and Hood, 2001). A consequence of this increased UDPGT and sulfotransferase activity is not only the increased elimination of xenobiotics, but also the increased conjugation and subsequent elimination of T3/T4, resulting in decreased serum thyroid hormone levels. As part of a feedback mechanism, the pituitary increases its secretion of TSH in order to stimulate the thyroid gland to increase production of thyroid hormones to restore homeostasis. A sustained increase in thyroid hormone production is often achieved through hypertrophy and proliferation of thyroid follicular cells. After chronic exposure to a threshold dose of a UDPGT substrate, hyperplasia and eventually tumors may develop in the thyroid gland due to sustained over-stimulation by TSH (Hiasa *et al.*, 1982; McClain *et al.*, 1988; Dellarco *et al.*, 2006;).

Based on these data, the following key events for thyroid tumor MOA were proposed by the Registrant, and the new mechanistic studies were conducted to support the proposed key events.

ii. Key Events for UDPGT-Mediated Thyroid MOA

(1) UDPGT upregulation
(2) Increased serum T3/T4 clearance
(3) Increased TSH levels. Reversible upon discontinuance of treatment
(4) Increased thyroid cell proliferation. Reversible upon discontinuance of treatment.

The thyroid MOA study (MRID 48604553) was conducted to investigate the mode of action for the increased incidence of thyroid follicular cell adenomas observed in male Han Wistar rats at the high dose level (600 ppm) observed in the 2 year carcinogenicity study conducted with benzovindiflupyr. The thyroid MOA study evaluated the effects of benzovindiflupyr treatment on a number of liver and thyroid parameters. Groups of 60 male Han Wistar Crl: WI (Han) rats were assigned to the main study and fed diets containing 0, 100, 600 or 1200 ppm (0, 9.9, 57.7, and 112.8 mg/kg/day) of benzovindiflupyr for a period of up to 14 consecutive days. The control group received blank diet for the same duration as the treated animals. An additional group of 60

male rats were fed diets containing phenobarbital sodium salt at 1200 ppm to act as a positive control for the liver and thyroid parameters investigated. Two additional groups of 15 male rats were assigned to a recovery study and dosed at 0 or 1200 ppm benzovindiflupyr for 14 consecutive days followed by a 63 day recovery period. The following were assessed at predetermined intervals from pre-trial until study completion for all animals: viability, clinical observations, body weights, and food and water consumption. Blood samples were also collected from all animals at each scheduled euthanasia time-point for thyroid function testing. All main study animals were terminated after completion of 1, 3, 7 or 14 days of treatment (i.e. days 2, 4, 8 or 15) and all recovery study animals were terminated after a subsequent 63 day recovery period (Day 78). Each animal underwent a detailed necropsy examination, and the liver and thyroid glands were weighed. The liver, thyroid glands and gross abnormalities from all animals were subjected to histological evaluation. 5-Bromo-2'- deoxyuridine (BrdU) was administered to each animal two hours before termination, and cell proliferation in the thyroid by BrdU labelling index was measured. Frozen samples of the liver from each animal were analyzed for hepatic microsomal protein content and UDPGT activity with thyroxine (T4) as substrate.

- **Key Event #1: UDP-Glucuronosyltransferase (UDPGT) Upregulation**

The first key event is upregulation of UDPGT activity by benzovindiflupyr dietary exposure. Table 7 shows that measured per mg protein or per grams liver, UDPGT activity was increased at doses ≥600 ppm (57.7 mg/kg/day). UDPGT activity measured relative to liver weight and body weight, activity was increased in all treatment groups after 3 days of treatment. In the recovery groups, UDPGT activity decreased to levels comparable to controls and typically lower than control values, but not to a statistically significant degree. In the positive control PB group, activity was increased after 1 day of treatment and increased at all time points.

Table 7: UDPGT Activity Results.					
	Dietary Concentration of Benzovindiflupyr (ppm)				PB (ppm)
Day	0	100	600	1200	1200
pmol/min/mg protein					
1	28.1 ± 7.65	28.6 ± 6.19	25.8 ± 4.50	30.0 ± 7.40 (↑7)	**32.8 ± 5.96 (↑17)**
3	28.3 ± 6.50	34.5 ± 7.47	**36.7 ± 6.34* (↑30)**	**42.6 ± 10.32** (↑51)**	**50.1 ± 12.75** (↑77)**
7	33.8 ± 5.12	33.5 ± 7.93	31.4 ± 8.83 (↓7)	**42.2 ± 9.44* (↑25)**	**52.6 ± 10.93** (↑56)**
14	22.1 ± 3.76	20.2 ± 3.93	24.4 ± 5.90	**33.1 ± 4.21** (↑50)**	**43.5 ± 9.09** (↑97)**
Recovery	23.6 ± 8.45	-	-	21.5 ± 7.59 (↓9)	
pmol/min/g liver					
1	971 ± 375.4	1017 ± 294.6	845 ± 190.4 (↓13)	**1081 ± 496.8 (↑11)**	**1191 ± 302.0 (↑23)**
3	968 ± 238.5	1238 ± 295.1	**1333 ± 284.2* (↑38)**	**1695 ± 522.1** (↑75)**	**2067 ± 518.9** (↑114)**
7	1171 ± 232.0	1200 ± 336.7	1224 ± 418.2	**1853 ± 516.2** (↑58)**	**2417 ± 557.0** (↑106)**
14	747 ± 166.9	770 ± 168.1	**1051 ± 329.1** (↑41)**	**1555 ± 262.6** (↑108)**	**2134 ± 549.8** (↑186)**
Recovery	848 ± 345.1			748 ± 243.7 (↓12)	
nmol/min/liver weight					
1	9.24 ± 4.045	9.51 ± 3.127	6.99 ± 1.309 (↓24)	8.15 ± 3.265 (↓12)	**10.81 ± 2.943 (↑17)**
3	8.97 ± 2.531	**12.42 ± 2.905** (↑39)**	**12.43 ± 3.116** (↑39)**	**17.06 ± 6.467** (↑90)**	**21.41 ± 5.741** ↑139)**
7	12.24 ± 2.389	12.66 ± 4.405	13.21 ± 4.685 (↑8)	**19.39 ± 4.681** (↑58)**	**29.80 ± 6.390** ↑143)**
14	8.19 ± 1.431	8.80 ± 2.165 (↑7)	**12.16 ± 3.584** (↑48)**	**17.22 ± 3.046** (↑110)**	**31.43 ± 9.600** ↑284)**
Recovery	11.02 ± 4.440			9.80 ± 3.101 (↓11)	
nmol/min/kg body weight					
1	48.85 ± 20.840	50.33 ± 15.040	38.07 ± 7.256 (↓22)	45.43 ± 18.282 (↓7)	**57.20 ± 15.410 (↑17)**
3	44.03 ± 10.343	**60.05 ± 14.735** (↑36)**	**66.52 ± 17.620** (↑51)**	**86.89 ± 29.756** (↑97)**	**110.53 ± 34.358** (↑151)**
7	52.59 ± 8.958	56.62 ± 18.282	**59.69 ± 21.090 (↑14)**	**96.99 ± 32.342** (↑84)**	**134.92 ± 29.614** (↑157)**
14	32.27 ± 6.801	34.47 ± 8.326	**49.82 ± 14.596** (↑54)**	**76.43 ± 12.507** (↑137)**	**122.44 ± 34.067** (↑279)**
Recovery	28.48 ± 10.419			25.49 ± 7.461 (↓10)	

[a] Data were obtained from pages 434, 436, 438, and 440 of MRID 48604586.

PB = Phenobarbital Sodium Salt

* Statistically significant difference from control group mean, $p<0.05$ (Dunnett's test)

** Statistically significant difference from control group mean, $p<0.01$ (Dunnett's test)

Table 8 presents data on hepatic microsomal protein content. As time increased there was a consistent increase in severity at a constant dose and it required a lower dose for the same change in protein content. After 14 days of treatment, hepatic microsomal protein content was increased in all treated groups. In the recovery group, there was no effect on hepatic microsomal protein content.

Table 8: Hepatic Microsomal Protein Content.

	Dietary Concentration of Benzovindiflupyr (ppm)				PB (ppm)
Day	**0**	**100**	**600**	**1200**	**1200**
1	33.8 ± 4.85	35.1 ± 4.22	32.6 ± 3.39	35.0 ± 9.58	35.9 ± 3.68
3	34.2 ± 2.71	35.8 ± 1.96 (↑5)	36.2 ± 3.09 (↑6)	**39.4 ± 4.12** (↑15)**	**41.4 ± 2.95** (↑21)**
7	34.5 ± 3.26	35.6 ± 2.84 (↑3)	**38.6 ± 2.77** (↑12)**	**43.7 ± 5.12** (↑27)**	**45.8 ± 2.73** (↑33)**
14	33.7 ± 2.83	**38.1 ± 3.87* (↑13)**	**42.7 ± 4.87** (↑27)**	**47.0 ± 5.33** (↑39)**	**48.9 ± 5.60** (↑45)**
Recovery	35.5 ± 3.57	-	-	35.0 ± 2.73 (↓1)	-

[a] Data were obtained from pages 433, 435, 437, and 439 of MRID 48604586.
PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, $p<0.05$ (Dunnett's test)
** Statistically significant difference from control group mean, $p<0.01$ (Dunnett's test)

Liver weight data are shown in Table 9 (absolute) and Table 10 (relative). After one day of treatment, the 1200 ppm animals had decreased absolute and relative liver weights compared to the concurrent controls. Animals in the 600 ppm dose group had decreased absolute weights following one day of treatment, but this was considered secondary to body weight decreases. Absolute liver weights were increased in positive control animals on Days 4, 8, and 15. Relative liver weights were increased in 1200 ppm benzovindiflupyr and positive control groups on Days 4, 8, and 15 and at 600 ppm on Day 15 (Table 10).

Table 9: Absolute Liver Weights.

Dose Group	Day				
	2	**4**	**8**	**15**	**63**
0 ppm	9.44 ± 0.89	9.19 ± 0.72	10.51 ± 1.24	11.12 ± 1.29	13.09 ± 1.62
100 ppm	9.29 ± 0.91	10.10 ± 1.15	10.40 ± 1.06	11.43 ± 1.19	
600 ppm	**8.37 ± 0.94* (↓11)**	9.32 ± 0.83	10.89 ± 1.14	11.73 ± 1.60	
1200 ppm	**7.73 ± 1.05** (↓18)**	9.98 ± 1.14	10.47 ± 1.39	11.09 ± 0.90	13.12 ± 1.48
1200 ppm PB	9.07 ± 0.94 (↓4)	**10.36 ± 1.03** (↑13)**	**12.45 ± 1.24** (↑18)**	**14.75 ± 1.90** (↑33)**	

PB = Phenobarbital Sodium Salt, MRID 48604553 pages 63-77
* Statistically significant difference from control group mean, $p<0.05$
** Statistically significant difference from control group mean, $p<0.01$

Table 10: Relative Liver Weights.					
Dose Group	**Day**				
	2	**4**	**8**	**15**	**63**
0 ppm (control)	5.002 ± 0.409	4.554 ± 0.271	4.518 ± 0.257	4.342 ± 0.283	3.426 ± 0.298
100 ppm Benzovindiflupyr	4.934 ± 0.191	4.857 ± 0.390	4.677 ± 0.349	4.473 ± 0.422	
600 ppm Benzovindiflupyr	4.548 ± 0.351	4.984 ± 0.464	4.894 ± 0.365	**4.779 ± 0.335 (↑10)**	
1200 ppm Benzovindiflupyr	**4.290 ± 0.330 (↓14)**	**5.120 ± 0.492 (↑12)**	**5.213 ± 0.514 (↑15)**	**4.931 ± 0.378 (↑14)**	3.445 ± 0.260
1200 ppm PB	4.790 ± 0.296	**5.296 ± 0.438 (↑16)**	**5.616 ± 0.478 (↑24)**	**5.748 ± 0.590 (↑32)**	

PB = Phenobarbital Sodium Salt, MRID 48604586 pages 63-77
* Statistically significant difference from control group mean, $p<0.05$
** Statistically significant difference from control group mean, $p<0.01$

As shown in Table 11, there were increases in hepatocellular hypertrophy at ≥600 ppm on Days 8 and 15, which returned to normal during the recovery period. In the positive control animals, hepatocellular hypertrophy was increased by Day 4. There were no changes in recovery animals.

Table 11: Hepatocyte Hypertrophy (microscopic).					
Dose Group	**Day**				
	2	**4**	**8**	**15**	**63**
0 ppm (control)	0	0	0	0	0
100 ppm Benzovindiflupyr	0	0	0	0	-
600 ppm Benzovindiflupyr	0	0	**10****	**13****	-
1200 ppm Benzovindiflupyr	0	0	**15****	**15****	0
1200 ppm PB	0	**13****	**15****	**15****	-

Values are total incidence out of 15 animals per dose per time interval. MRID 48604553 pages 37-38
PB = Phenobarbital Sodium Salt
- = Not determined
* Statistically significant difference from control group mean, $p<0.05$ Fisher's Exact Test
** Statistically significant difference from control group mean, $p<0.01$ Fisher's Exact Test

The CARC concluded that the Key Event #1 of UDPGT upregulation at doses >600 ppm is supported by the submitted data.

- **Key Event #2: Increased serum T3/T4 clearance and consequently decreased circulating T3/T4 Levels**

T3 and T4 are inactivated in the liver mainly by UDPGT-mediated conversion to glucuronide derivatives, which are eliminated via urine and bile (Rutgers *et al.*, 1989). This results in reduced serum T4 concentrations in both mice (Hood *et al.*, 2003) and rats (Hood *et al.*, 1999; Liu *et al.* 1995). The activation of these Phase II enzymes is directly responsible for the cascade of events producing lower serum T3/T4, increased TSH, elevated thyroid follicular cell proliferation, and eventually thyroid tumors (Barter and Klaassen, 1992; Hurley *et al.*, 1998; Dellarco *et al.*, 2006). The activities of UDPGT was demonstrated to be elevated with benzovindiflupyr oral exposure.

As shown in Table 12, serum T3 hormone values were decreased at all-time points in the 1200 ppm benzovindiflupyr and PB positive control groups. At 600 ppm, T3 was decreased on Days 2 and 15. In the 100 ppm group, T3 values were decreased pm Day 15. There were no differences from control in the recovery group previously treated with 1200 ppm benzovindiflupyr.

Table 12: Serum T3 Levels (ng/dL).

	Dietary Concentration of Benzovindiflupyr (ppm)				PB (ppm)
Day	**0**	**100**	**600**	**1200**	**1200**
2	138 ± 9.5	126 ± 15.4(↓9)	**107 ± 17.3** (↓22)**	**95 ± 15.2** (↓31)**	**94 ± 12.1** (↓32)**
4	119 ± 15.2	124 ± 21.3	111 ± 21.0(↓7)	**85 ± 16.0** (↓21)**	**93 ± 13.2** (↓21)**
8	118 ± 25.3	121 ± 26.1	107 ± 14.4(↓9)	**103 ± 16.6(↓13)**	**101 ± 25.3(↓14)**
15	119 ± 15.3	**103 ± 19.5* (↓13)**	**92 ± 20.5** (↓23)**	**78 ± 16.3** (↓34)**	**85 ± 20.1** (↓29)**
78	84 ± 16.8	-	-	90 ± 13.2	

[a] Data were obtained from pages 192 and 195 of MRID 48604553.
PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, $p<0.05$ (Dunnett's test)
** Statistically significant difference from control group mean, $p<0.01$ (Dunnett's test)

As shown in Table 13, serum T4 hormone levels were decreased pm Days 2, 4, and 15 in 1200 ppm with benzovindiflupyr and PB positive control groups (Table 13). There were no differences from control in the recovery group treated with 1200 ppm benzovindiflupyr.

Table 13: Serum T4 Levels (ng/dL).					
Day	Dietary Concentration of Benzovindiflupyr (ppm)				PB (ppm)
	0	100	600	1200	1200
2	5.8 ± 0.67	6.0 ± 0.50	5.6 ± 0.54	**5.1 ± 0.75** (↓12)**	**4.8 ± 0.60** (↓17)**
4	5.8 ± 0.64	5.7 ± 0.76	5.8 ± 0.97	**4.6 ± 0.82** (↓21)**	**4.6 ± 0.57 (↓21)**
8	5.9 ± 0.91	6.4 ± 0.92	6.4 ± 1.20	6.2 ± 0.90	5.9 ± 1.04
15	6.0 ± 0.94	5.8 ± 0.74	5.6 ± 0.49 (↓7)	**5.4 ± 0.93 (↓10)**	**5.0 ± 0.39** (↓17)**
78	4.8 ± 0.60	-	-	5.2 ± 0.64	

[a] Data were obtained from pages 193 and 195 of MRID 48604586.
PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, $p<0.05$ (Dunnett's test)
** Statistically significant difference from control group mean, $p<0.01$ (Dunnett's test)

The CARC concluded that the Key Event #2 of increased T3/T4 clearance at doses >600 ppm is supported by the submitted data.

- **Key Event #3: Increased TSH Levels**

The first two key events leading to thyroid alterations occur in the liver; however, reduced circulating levels of T3/T4 can activate the hypothalamic-pituitary-thyroid axis feedback mechanism in an attempt to maintain thyroid hormone homeostasis. Serum T4 and T3 concentrations are monitored by the hypothalamus and the anterior pituitary gland. A decrease in serum thyroid hormone concentrations stimulates the hypothalamus to secrete TRH, which then stimulates the release of TSH from the anterior pituitary. Increased pituitary secretion of TSH stimulates the function and the growth of the thyroid gland, resulting in an increased production of thyroid hormones, T4 and T3, to restore thyroid hormone homeostasis.

As shown in Table 14, serum TSH values were decreased on Day 2 at 1200 ppm and PB groups. On Day 15, TSH values were increased at doses ≥600 ppm of benzovindiflupyr and in the positive control PB group. There were no differences from control in the recovery group previously treated with 1200 ppm benzovindiflupyr.

Table 14: Serum TSH Levels (ng/dL).					
Day	Dietary Concentration of Benzovindiflupyr (ppm)				PB (ppm)
	0	100	600	1200	1200
2	5.3 ± 1.64	4.9 ± 1.74	4.8 ± 1.61	**4.7 ± 1.47 (↓11)**	**4.7 ± 1.38 (↓11)**
4	3.1 ± 1.52	3.9 ± 1.66	3.7 ± 1.41	3.0 ± 1.75	3.1 ± 1.19
8	5.4 ± 1.40	6.1 ± 3.84	5.7 ± 3.06	4.8 ± 1.90	5.8 ± 2.16
15	4.6 ± 1.96	4.3 ± 1.34	**6.0 ± 3.52 (↑30)**	**7.5* ± 4.41 (↑63)**	**7.8** ± 3.40 (↑70)**
78	4.5 ± 1.66	-	-	4.9 ± 2.36 (↑9)	

[a] Data were obtained from pages 194 and 195 of MRID 48604586. PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, $p<0.05$ (Dunnett's test)
** Statistically significant difference from control group mean, $p<0.01$ (Dunnett's test)

The CARC concluded that the Key Event #3 of increased TSH levels at doses >600 ppm is supported by the submitted data.

- **Key Event #4: Increased Thyroid Weight and Cell Proliferation**

The data presented previously showed that exposure to benzovindiflupyr results in decreases in serum thyroid hormones, T3/T4, which results in increased TSH levels. Increases in TSH levels results in increased thyroid weight and follicular cell proliferation. This process of thyroid follicular cell proliferation, if sustained for a prolonged period of time, can result in thyroid gland tumors (McClain, 1992). The hypothesis of sustained proliferation leading to tumorigenesis is similar to that described for the liver.

As shown in Table 15, absolute thyroid weights were increased at 1200 ppm Day 15 and in the positive control group (1200 ppb PB) on Day 8.

Table 15: Absolute Thyroid Weights (g).

Dose Group	Day				
	2	4	8	15	63
0 ppm (control)	0.0137 ± 0.0023	0.0151 ± 0.0039	0.0148 ± 0.0033	0.0141 ± 0.0026	0.0191 ± 0.0043
100 ppm Benzovindiflupyr	0.0140 ± 0.0027	0.0146 ± 0.0035	0.0149 ± 0.0022	0.0164 ± 0.0039	
600 ppm Benzovindiflupyr	0.0116 ± 0.0027	0.0118 ± 0.0030*	0.0151 ± 0.0026	0.0143 ± 0.0028	
1200 ppm Benzovindiflupyr	0.0133 ± 0.0022	0.0135 ± 0.0031	0.0149 ± 0.0027	**0.0162 ± 0.0037 (↑15)**	0.0180 ± 0.0041
1200 ppm PB	0.0126 ± 0.0022	0.0143 ± 0.0029	**0.0167 ± 0.0031 (↑13)**	**0.0178 ± 0.0030** (↑26)**	

PB = Phenobarbital Sodium Salt, MRID 48604553 pages 63-77

* Statistically significant difference from control group mean, $p<0.05$

** Statistically significant difference from control group mean, $p<0.01$

As shown in Table 16, relative thyroid were significantly increased on Days 8 and 15 at 1200 ppm benzovindiflupyr and the positive control groups.

Table 16: Relative Thyroid Weights (g)

Dose Group	Day				
	2	4	8	15	63
0 ppm (control)	0.00727 ± 0.00116	0.00743 ± 0.00162	0.00638 ± 0.00137	0.00552 ± 0.00087	0.00503 ± 0.00124
100 ppm Benzovindiflupyr	0.00744 ± 0.00154	0.00705 ± 0.00181	0.00671 ± 0.00105	0.00640 ± 0.00146	
600 ppm Benzovindiflupyr	0.00630 ± 0.00124	0.00631 ± 0.00169	0.00677 ± 0.00110	0.00585 ± 0.00109	

1200 ppm Benzovindiflupyr	0.00739 ± 0.00103	0.00693 ± 0.00141	**0.00740 ± 0.00106 (↑16)***	**0.00721 ± 0.00162 (↑31)****	0.00476 ± 0.00120
1200 ppm PB	0.00669 ± 0.00134	0.00731 ± 0.00158	**0.00754 ± 0.00143 (↑18)***	**0.00699 ± 0.00133 (↑27)****	

PB = Phenobarbital Sodium Salt, MRID 48604553 pages 63-77
* Statistically significant difference from control group mean, $p<0.05$
* *Statistically significant difference from control group mean, $p<0.01$

As shown in Table 17, changes to the thyroid were limited to follicular cell hypertrophy in positive PB control animals on Day 15 at the short exposure durations. There were no differences from control in the recovery group treated with 1200 ppm benzovindiflupyr.

Table 17: Follicular Cell Hypertrophy.

Dose Group	Day				
	2	**4**	**8**	**15**	**63**
0 ppm (control)	0	0	0	0	0
100 ppm Benzovindiflupyr	0	0	0	0	-
600 ppm Benzovindiflupyr	0	0	0	0	-
1200 ppm Benzovindiflupyr	0	0	0	0	0
1200 ppm PB	0	0	0	**8****	-

Values are total incidence out of 15 animals per dose per time interval. MRID 48604586 page 15
PB = Phenobarbital Sodium Salt
- = Not determined
* Statistically significant difference from control group mean, $p<0.05$ Fisher's Exact Test
** Statistically significant difference from control group mean, $p<0.01$ Fisher's Exact Test

As shown in Table 18, follicular cell hypertrophy also occurred after longer exposure at doses of 750 ppm solatenol or above on Day 29 or on Days 8, 15, and 29 at 1500 ppm The thyroid tumors were first evident at week 97 of the chronic rat study, much longer solatenol exposure durations than this study. The overall data shows that while some of the early key events can occur within 15 days, the follicular cell hypertrophy takes 29 days for development at doses less than 1200 ppm solatenol and 1500 ppm solatenol is necessary to induce follicular cell hypertrophy to occur in the shorter timeframes.

Table 18: Follicular Cell Hypertrophy: Higher Doses and Longer Duration*.

Dose	Thyroid follicular cell hypertrophy, diffuse, minimal			
	Day 4	**Day 8**	**Day 15**	**Day 29**
0 ppm	0/5	0/5	0/5	0/4
100 ppm	0/5	0/5	0/5	0/5
750 ppm	0/5	0/5	0/5	**2/4**
1500 ppm	0/5	**1/5**	**2/5**	**1/5**

* Data were obtained from pages 18 of MRID 48604586.

As shown in Table 19, thyroid cell proliferation, as measured by S-phase labelling, was inconclusive in the first 8 days of the study, as there was a statistically significant increase in labelling in the low-dose animals, without dose-response. By Day 15 sampling, S-phase labelling was increased at1200 ppm benzovindiflupyr and in PB positive controls and the increases at Day 15 were correlated with thyroid hormone, organ weight and histological changes. There were no changes to S-phase labelling in the recovery animals.

Table 19: Thyroid Cell Proliferation Measurements.

Day	Dietary Concentration of Benzovindiflupyr (ppm)				PB (ppm)
	0	100	600	1200	1200
1	1.29 ± 0.29	2.21 ± 0.29** (↑71)	1.81 ± 0.62 (↑40)	1.61 ± 0.54 (↑25)	2.40 ± 0.93 (↑86)
3	1.03 ± 0.39	**2.25 ± 0.46** (↑118)**	**2.78 ± 0.69** (↑170)**	1.34 ± 0.50 (↑30)	1.56 ± 0.87* (↑51)
7	0.69 ± 0.26	**2.47 ± 0.62** (↑258)**	0.87 ± 0.32 (↑26)	**1.88 ± 0.84** (↑172)**	1.62 ± 0.60** (↑135)
14	0.46 ± 0.21	0.70 ± 0.35	0.60 ± 0.19 (↑30)	**2.18 ± 0.69** (↑374)**	**2.15 ± 0.80** (↑367)**
Recovery	0.36 ± 0.11	-	-	0.30 ± 0.09 (↓17)	-

[a] Data were obtained from page 467 of MRID 486045553
PB = Phenobarbital Sodium Salt
* Statistically significant difference from control group mean, $p<0.05$ (Dunnett's test)
** Statistically significant difference from control group mean, $p<0.01$ (Dunnett's test)

It is postulated that increased TSH levels should result in increased thyroid weights, followed by follicular cell proliferation and (if sustained for a prolonged period of time) can result in thyroid gland tumors. In the case of benzovindiflupyr, TSH levels were increased at doses ≥600 ppm on Day 1. The increase in TSH did not correlate with increased thyroid weights at 600 ppm (the tumorigenic dose). However, increases in absolute and relative thyroid weights were seen at a higher dose (1200 ppm). It is noted that thyroid weights are difficult to measure accurately, being prone to trimming errors, weighed concurrently with the parathyroids and typically being weighed after fixation. Also, thyroid weight is considered an associative, not a key event for thyroid adenoma formation. On the other hand, thyroid follicular cell proliferation, which is considered a key event when establishing the mode of action, was observed at the tumorigenic dose. Furthermore, thyroid follicular cell hypertrophy was also observed at the tumorigenic dose.

The CARC concluded that the Key Event #4 of increased thyroid cell proliferation at the tumorigenic dose of 600 ppm was supported by the submitted data.

In summary, the MOA for benzovindiflupyr-induced rodent thyroid effects is similar to that of phenobarbital. Benzovindiflupyr and phenobarbital upregulated UDPGT activity, which caused increased T3/T4 clearance. Compensatory increased TSH production led to increased thyroid cell proliferation.

iii. Strength, Consistency, and Specificity of Association of Tumor Response with Key Events

In the available mechanistic data, the key events observed following exposure to benzovindiflupyr in the rat occurred in a biologically relevant temporal sequence, were dose-dependent, and took place at dose levels that were at or below the doses that produced tumors. The studies underlying the Key Events in the MOA were conducted using thyroid tissue or plasma from orally dosed animals and the thyroid hormones measured are specific to the thyroid. All of these key events were identified, characterized in terms of dose and temporal response, and were shown to be reversible. This supports that induction of UDPGT is the initial key event causing the thyroid tumors in male rats. Therefore, this MOA is considered to have adequate strength, consistency, and specificity.

iv. Biological Plausibility and Coherence

As shown in Table 20, the proposed MOA is considered biologically plausible and coherent, as it is a known MOA for thyroid tumorigenesis in rodents. The common initial key event is upregulation of UDPGT that leads to increased hepatic metabolizing enzyme activities. In male rats, the data showed that oral benzovindiflupyr exposure led to increase in hepatic Phase II enzyme activities, which resulted in another series of events leading to thyroid follicular cell proliferation. The early key events of hepatic enzyme induction and cellular (liver and thyroid) proliferation, as well as the associative events of increased liver weight and hepatocellular hypertrophy, were largely reversible on cessation of treatment.

Table 20. Analysis of Benzovindiflupyr Thyroid Tumor MOA.

Key Event #1: UDPGT Phase II liver enzyme induction Key Event #2: Decreased T_3/T_4 Key Event #3. Increased TSH Key Event #4. Thyroid follicular cell proliferation and increased thyroid weight Key events 1, 2, 3, & 4 are reversible	
Strength of	+
Consistency of association	+
Specificity of association	+
Dose response concordance	+
Temporal relationship	+
Coherence & plausibility	+ & +

+: Attribute present

v. Alternative Modes of Action for Thyroid Tumors

The following alternative MOAs may be considered for thyroid tumor formation:

- **DNA reactivity**

Based on the available genotoxicity data, benzovindiflupyr and its metabolites tested negative in all genotoxicity assays. Therefore, genotoxicity MOAs are not likely.

- **Inhibition of the active transport of inorganic iodide into the follicular cell (iodide pump).**

No data were available.

- **Inhibition of thyroid peroxidase that converts inorganic iodide into organic iodide. And couples iodinated tyrosyl moieties into thyroid hormone.**

A rat thyroid peroxidase study (MRID 48604555) demonstrated that benzovindiflupyr does not inhibit thyroid peroxidase. A summary of the data are presented below in Table 21.

Table 21: Rat Thyroid Peroxidase Assay Results.

Addition (a)	Thyroid peroxidase activity (nmol/min/mg protein) (b)	Percentage of control values
Control (DMSO only)	1.03 ± 0.057	100
Benzovindiflupyr, 0.01 µM	1.08 ± 0.092	105
Benzovindiflupyr, 0.1 µM	1.08 ± 0.106	105
Benzovindiflupyr, 1 µM	0.92 ± 0.046	89
Benzovindiflupyr, 10 µM	1.03 ± 0.111	100
PTU, 10 µM	0.0 ± 0.00***	0

(a) Benzovindiflupyr and PTU were added in DMSO (2.5 µL/incubation). MRID 48604555 page 12

(b) results are presented as mean ±SD for triplicate incubations

*** significantly different from control $p<0.001$

- **Inhibition of thyroid hormone release into the blood.**

Direct thyroid gland effects and are not supported by the available data on benzovindiflupyr. The clear evidence of an increased T3/T4 clearance by UDPGT upregulation does not favor a direct effect of benzovindiflupyr on thyroid hormone biosynthesis and release. Moreover, mechanistic studies using rat thyroid microsomes showed benzovindiflupyr did not affect thyroid peroxidase (MRID 48604555).

- **Damage to thyroid follicular cells.**

Damage to thyroid follicular cells is not supported by the available data, as histopathology data on the thyroid gland of rats do not show overt cytotoxicity (MRID 48604556).

- **Inhibition of the conversion of T4 to T3 by 5'-monodeiodinase at various sites in the body.**

No data were available.

- **Enhancement of the metabolism and excretion of thyroid hormone by the liver, largely through the action of UDPGT.**

The available data indicate that the proposed MOA (enhancement of the metabolism and excretion of thyroid hormone by the liver, largely through induction of UDPGT enzymes) is plausible and the most likely mechanism leading to thyroid tumors at high doses in the male rat. Benzovindiflupyr increased the activity of UDPGTs, which catabolize T_3/T_4.

vi. Data Limitations, Uncertainties, and Inconsistencies

No data limitations, uncertainties, or inconsistencies were noted.

vii. Human Relevance

While the established mode of action of benzovindiflupyr for follicular thyroid carcinogenesis in animals is qualitatively plausible in humans, it is quantitatively implausible based on differences in thyroid physiology (also reflected in differences in incidence data) between rats and humans. "*Quantitatively*, if humans develop cancer through thyroid-pituitary disruption, it appears that humans are less sensitive to the carcinogenic effects than are rodents. Rodents show significant increases in cancer with thyroid-pituitary disruption; humans show little, if any" (USEPA 1998).

VII. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

Benzovindiflupyr was administered in the diet at dose levels of 0, 1.21, 4.88 and 30.17 mg/kg/day in males and 0, 1.65, 6.66 and 27.44 mg/kg/day for females for 104 weeks. There was no evidence of carcinogenicity in female rats. In male rat at the high dose, there was a statistically significant trend and pair-wise significance ($p<0.05$) for increased thyroid follicular cell adenomas and the combined follicular cell adenomas and/or carcinomas. The incidences for adenomas (18%) exceeded the historical control range (2-11%) of the testing laboratory. The CARC concluded that the thyroid follicular cell tumors (adenomas and the combined

adenomas/carcinomas) are treatment-related, since the incidences were statistically significantly increased when compared to the concurrent controls and exceeded the historical control range.

Male rats at the high dose also had increased incidence of granular cell tumors of the brain (10%) when compared to controls (4%). Although there was no pair-wise significance, the CARC determined that the brain tumors are treatment-related since there was a positive trend ($p<0.01$) and the incidences exceeded the historical control range (0-6%). The Committee noted that the tumors occurred late in the study (first tumor was seen at Study Week 91). The CARC determined that the dose levels tested were adequate to assess the carcinogenic potential of solatenol in rats, based on the presence of systemic toxicity (decrements in body weights and clinical signs in both sexes) and the occurrence of tumors at the high dose.

There was no evidence of carcinogenicity in male and female CD-1 mice fed diets containing solatenol at 0, 20, 60, or 200 ppm (equivalent to 0, 2.62, 7.55 and 26.18 mg/kg/day for males and 0, 2.89, 8.67 and 29.26 for females) for 18 months. The CARC determined that the doses tested were adequate to assess the carcinogenic potential of solatenol, based on clinical signs of toxicity (piloerection and rolling gain) seen in females at the high dose and the presence of hyperplasia of the colon and cecum in both sexes of mice at the high dose.

There was no evidence of mutagenicity *in vivo* or *in vitro*.

The CARC determined that the non-mutagenic mode of action for thyroid follicular cell tumor was adequately supported by studies that clearly identified the sequence of key events, dose-response relationship and temporal concordance for thyroid follicular cell tumors. Key events leading to the progression towards thyroid tumors included sequentially induction of phase II hepatic enzymes (UDPGT), resulting in increased serum T3/T4 clearance, increased TSH levels, increased thyroid cell proliferation, and increased thyroid cell hyperplasia, which eventually result in the formation of thyroid tumors. Alternate modes of actions were also considered, but the committee determined they were not supported by the available data.

VIII. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the Agency's 2005 Guidelines for Carcinogen Risk Assessment, benzovindiflupyr is classified as showing "*Suggestive Evidence of Carcinogenic Potential*", based on brain granular cell tumors in male rats at the high dose only. A non-genotoxic mode of action for thyroid follicular cell tumors observed in male rats has been established as a result of upregulation of UDPGT, increased clearance of T3 and T4 hormones and increased TSH levels resulting in increased thyroid cell proliferation, which progresses to form thyroid tumors. There was no evidence of carcinogenicity in female rats or in male or female mice. In addition, there is no concern for mutagenicity.

IX. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Quantification of carcinogenicity is not required. When there is suggestive evidence of carcinogenicity, the Agency does not attempt a dose-response assessment as the nature of the data generally would not support one. Therefore, the Agency has determined that quantification of risk using a non-linear approach (i.e., RfD) will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to benzovindiflupyr.

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